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MEK inhibitor trametinib in combination with gemcitabine regresses a patient-derived orthotopic xenograft (PDOX) pancreatic cancer nude mouse model

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ABSTRACT

Pancreatic cancer is resistant to treatment and needs precision individualized therapy to improve the outcome of this disease. Previously, we demonstrated that trametinib (TRA), a MEK inhibitor, could inhibit a pancreatic cancer patient-derived orthotopic xenograft (PDOX). In the present study, we show that gemcitabine (GEM) in combination with TRA was more effective than TRA alone. We implanted a patient pancreatic cancer orthotopically in the pancreatic tail of nude mice to establish the PDOX model. After seven weeks of tumor growth, we divided 32 pancreatic-cancer PDOX nude mice into 4 groups of eight: untreated control; GEM (once a week for 2 weeks); TRA (14 consecutive days); GEM + TRA (GEM: once a week for 2 weeks, TRA:14 consecutive days). We found that treated mice on day 14 had significantly reduced tumor volume in comparison to untreated control. TRA and the combination of GEM + TRA therapy significantly inhibited tumor development in comparison to GEM alone. However, GEM + TRA inhibited the PDOX tumor growth significantly greater than TRA alone. These results suggest the clinical potential of the combination of TRA and GEM for pancreatic cancer.

1. Introduction

Pancreatic cancer is one of the most resistant cancers. Pancreatic cancer is the fourth major cause of cancer-related death in men and women in the Unites States. The American Cancer Society estimates that in 2018, approximately 55,440 people will be diagnosed with pancreatic cancer and about 44,330 people will die because of this cancer in the Unites States (American Cancer Society, 2018). It is projected that pancreatic cancer will become the second leading cause of cancer death in the United States by 2030 (Rahib et al., 2014). At present, the only potentially curative treatment for pancreatic cancer is surgery, which is effective for patients in their early stage. However, due to lack of early detection techniques for pancreatic cancer, most patients are first diagnosed when the cancer has already disseminated to distant sites. 5-fluorouracil-oxaliplatin-irinotecan (FOLFIRINOX) or gemcitabine (GEM) and NAB-paclitaxel are first line chemotherapy for pancreatic cancer (Conroy et al., 2011a; Von Hoff et al., 2013; Vogel et al., 2014); however, the 5-year survival rate is around 5%. Wang-Gillam et al. (2016) found that nanoliposomal irinotecan in combination with 5-fluorouracil and folinic acid could be used as second-line chemotherapy in patients with metastatic pancreatic cancer who were previously treated with GEM-based therapy. Different types of chemotherapeutic drugs have been given to these patients, but they show resistance to most of these therapies. Since pancreatic cancer is recalcitrant, there is an urgent need for precision individualized therapy to ameliorate conditions of this disease.

To accomplish this goal of precision, individualized treatment of cancer patients, our laboratory established the patient-derived orthotopic xenograft (PDOX) nude mouse model (Hoffman, 2017) employing the surgical orthotopic implantation (SOI) technique, which includes pancreatic (Hiroshima et al., 2014a; Fu et al., 1992; Hiroshima et al., 2014b; Hiroshima et al., 2015a) breast (Fu et al., 1993), ovarian (Fu

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and Hoffman, 1993), lung (Wang et al., 1992), cervical (Hiroshima et al., 2015b), colon (Fu et al., 1991; Metildi et al., 2014; Hiroshima et al., 2014c) stomach (Furukawa et al., 1993), melanoma (Kawaguchi et al., 2016a,b; Kawaguchi et al., 2017a,b; Yamamoto et al., 2016) and sarcoma (Murakami et al., 2016a; Hiroshima et al., 2015c; Kiyuna et al., 2016; Murakami et al., 2016b; Hiroshima et al., 2015d) Our PDOX model has several benefits compared to subcutaneous-transplant mouse tumor models (Hoffman, 2015).

In a recent pancreatic cancer PDOX study, cobimetinib (COB) and trametinib (TRA), both MEK inhibitors, were the only agents of 10 tested that caused tumor regression. The pancreatic cancer PDOX model therefore was very useful in identification of appropriate efficacy of the MEK inhibitors (Kawaguchi et al., 2017c).

In the present study, we show that TRA can overcome partial GEM resistance to significantly regress the pancreatic cancer PDOX model.

2. Materials and methods

2.1. Mice

In the present study, 4–6 weeks old, athymic *nu/nu* nude mice (AntiCancer Inc., San Diego, CA) were utilized. All experimental protocols and data collection were as previously described (Kawaguchi et al., 2016b, 2017a,b; Yamamoto et al., 2016; Murakami et al., 2016a; Hiroshima et al., 2015c; Kiyuna et al., 2016) Mice were housed in a barrier facility on a high efficiency particulate arrestance (HEPA)-filtered rack and put under standard conditions on a 12:12-h light:dark cycle. Mice were fed an autoclaved laboratory rodent diet. All mouse surgical processes and imaging were done with the mice anesthetized through subcutaneous injection of a ketamine mixture (0.02 ml solution of 20 mg/kg ketamine, 15.2 mg/kg xylazine, and 0.48 mg/kg acepromazine maleate). The reaction of mice throughout surgery was observed to assure sufficient depth of anesthesia. The mice were monitored every day and humanely sacrificed by CO_2 inhalation once they met these humane endpoint criteria: severe tumor burden (tumors of more than 20 mm in diameter), prostration, significant body weight loss, difficulty breathing, rotational motion, and body-temperature drop. All animal studies were performed with an AntiCancer, Inc. Institutional Animal Care and Use Committee (IACUC)-protocol approved for the present study and following the principles and procedures defined in the National Institutes of Health (NIH) Guide for the Care and Use of Animals under Assurance Number A3873-1 (Kawaguchi et al., 2016a,b; Kawaguchi et al., 2017a,b; Yamamoto et al., 2016; Hiroshima et al., 2015d), All procedures were performed as per applicable guidelines and regulations of the institutions involved in the present study.

2.2. Patient-derived tumor

The pancreatic cancer was resected in the Department of Surgery, University of California, San Diego (UCSD). All procedures were conducted as per relevant guidelines and regulations of the institution. Written informed consent was provided by the patient. The Institutional Review Board (IRB) (#140046CX) of UCSD approved this experiment (Kawaguchi et al., 2017c).

2.3. Establishment of the pancreatic cancer PDOX model by surgical orthotopic implantation (SOI)

All experimental protocols and data collection were as previously described (Kawaguchi et al., 2016b, 2017a; Kawaguchi et al., 2017b; Yamamoto et al., 2016; Murakami et al., 2016a; Hiroshima et al., 2015c; Kiyuna et al., 2016) Subcutaneously-grown tumors were collected. Then these tumors were cut into tiny pieces (3 mm^3). First nude mice were anesthetized using the ketamine solution as mentioned above. Then the pancreas was visualized by making a 1–2 cm skin incision on the left-side abdomen over the skin, fascia and peritoneum. Surgical sutures (8–0 nylon) were used to implant tumor pieces onto the tail of the pancreas to establish the PDOX model (Hiroshima et al., 2014a; Fu et al., 1992; Hiroshima et al., 2014b, 2015a) The wound was sealed using a 6–0 nylon suture (Ethilon; Ethicon, Inc., NJ, USA) (Kawaguchi et al., 2017c).

2.4. Treatment study design

All experimental protocols and data collection were as previously described (Kawaguchi et al., 2016b, 2017a,b; Yamamoto et al., 2016; Murakami et al., 2016a; Hiroshima et al., 2015c; Kiyuna et al., 2016) After seven weeks of tumor growth, 32 pancreatic-cancer PDOX nude mice were randomized into 4 categories of eight: untreated control; GEM (100 mg/kg, i.p., once a week for two weeks); TRA (0.3 mg/kg, p.o., 14 successive days); GEM + TRA (GEM: 100 mg/kg, i.p., once a week for two weeks, TRA: 0.3 mg/kg, p.o., 14 successive days). On day 14 all treated mice showed significantly reduced tumor growth in contrast to the untreated control ($p < 0.0001$, respectively).

2.5. Imaging of the pancreatic cancer PDOX model

Macroscopic tumors were imaged using the OV100 Small Animal Imaging System (Olympus, Tokyo, Japan) (Kawaguchi et al., 2017c).

2.6. Histological examination

All experimental protocols and data collection were previously described [4,5]. 10% formalin was used to fix all fresh tumor samples. Then fixed tumor samples were embedded in paraffin prior to section and staining. Paraffin-embedded tissue sections (5 μm) were placed in xylene (to eliminate paraffin) and then rehydrated in a graded ethanol series. Standard protocols were employed to perform hematoxylin and eosin (H&E) staining. Histological analysis was performed using a BHS System Microscope (Olympus Corporation, Tokyo, Japan). All images were obtained using INFINITY ANALYZE software (Lumenera Corporation, Ottawa, Canada) (Kawaguchi et al., 2016a,b; Kawaguchi et al., 2017a,b; Yamamoto et al., 2016; Hiroshima et al., 2015d).

2.7. Statistical analysis

All statistical analyses were done using JMP version 11.0. Significant differences for continuous variables were determined with the Mann-Whitney *U* test for efficacy of the combination of TRA and GEM on the pancreatic cancer PDOX. Data in the bar graphs are presented as mean and error bars show standard deviation (SD). P values ≤ 0.05 are considered statistically significant (Kawaguchi et al., 2017c).

3. Results

3.1. Efficacy of GEM and TRA on the pancreatic cancer PDOX

To test the efficacy of GEM and TRA monotherapy on the pancreatic cancer PDOX, we administered GEM (once a week for 2 weeks) and TRA (14 consecutive days). We found that monotherapy with GEM or TRA significantly reduced tumor volume in the pancreatic cancer PDOX in contrast to the untreated control (TRA: $p < 0.0001$; GEM: $p < 0.0001$) on day 14 after initiation of treatment. Although GEM inhibited tumor growth, the tumor still grew reflecting the limited efficacy of GEM in the clinic. In addition, the GEM + TRA combination was significantly more effective compared to TRA alone ($p = 0.014$) (Figs. 1 and 2). Our results demonstrate tumor regression in the PDOX mouse model, which is an important event, as it suggests potential clinical activity (Kurmasheva and Houghton, 2017).